Optimizing Acetylcholinesterase Production by Altering Cell Line and Media Composition

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Background

The Clark Lab designs nanosensors for chemical imaging in living systems, including a DNA-based nanosensor for mapping enzyme activity based upon this reaction time. Acetylcholinesterase (AChE) is a key component of this sensor, determining the neurotransmitter Acetylcholine by hydrolyzing it into inactive Acetyl-CoA and Choline. The enzymatic activity of AChE is thus crucial to the sensor's operation.

Adaptation

A steep reduction in Food and Drug Administration drugs leading to anti-cholinesterase activity, resulting in widespread cell death and a lack of attachment to culture dishes. To combat this, a gradual adaptation procedure was employed, wherein the percentage of FBS in the cell medium was reduced from 10% to 5% and eventually to 2%, with reductions occurring over the span of two weeks. The gradual adaptation allowed cells to adjust to the lack of attachment proteins in the environment and other cell-protein expressions accordingly. Upon initial adaptation, 2% FBS, low cell attachment, contact inhibition were noted. In contrast, 10% FBS showed clear improvements to cell attachment and proliferation, with the implementation of these dishes showing increased enzyme activity within 24 hours.

Transfection

In order to elicit AChE expression and secretion within a culture of CHO K1 cells, adherent HEK 293 cells were transfected with a recombinant plasmid. Additionally, a recombinant plasmid containing GFP was transfected into HEK cells in order to monitor transfection efficiency. The CHO K1 cells were then co-cultured with HEK 293T cells that were modified to produce and secrete recombinant AChE.

Results

To provide a basis for comparison between different culture conditions and cell lines, Ellman’s assay was used using a plate reader to measure the absorbance of waste media from cells in each condition every two hours. To quantify the maximum optical density in roughly half an hour, the well type CHO K1 cells were used to produce transfection medium to track the maximum value. This was followed by HEK 293T cells with 10%, 5%, and 2% FBS medium, and the wild type cells of both cell lines.

Discussion & Conclusion

The first and most notable result was an increased enzyme activity seen in transfected CHO K1 cells as opposed to HEK 293T cells under any conditions. This suggests that, in contrast to the cell line producing AChE in a gradient concentration of HEK 293T cells that was transfected with the same plasmid, transfected CHO 293T cells with 10% FBS medium showed a corresponding increase in AChE activity within 20 minutes. Further verification is needed to determine whether this trend is continued for the waste media of CHO K1 cells after purification, and to determine exactly how much enzyme activity for AChE is produced by CHO K1 culture.

The second major result from the experiment was an adenovirus purification of AChE from CHO K1T cells cultured in 7% FBS medium, producing a much lower enzyme activity than that seen for HEK 293T cells in 10% FBS medium. This underproduction could have resulted from a variety of factors. One possibility is a change in gene expression from the cells due to stress of adaptation or due to different environmental conditions. Another possibility is the removal of proteins within the FBS that contributed to the increased expression of the adenovirus AChE. Despite this decrease, 2% FBS medium did show benefit in reducing production time, with waste medium containing 10% FBS and 5% FBS showing a 22% and 31% increase in 2% FBS medium, likely due to the increased production of AChE in culture conditions.

Future work could test the trends seen in this project and expand upon the work of continuing adaptation, including further purifying adenovirus activity of purified waste media in order to discover the most effective CHO K1 cells. Additionally, the goal of optimization and maximization of AChE production could be further explored through the design of different plasmids, allowing for the expression of AChE production or secretion genes in the same suspension cultures in order to significantly increase the amount of cell medium collected from one culture and therefore the rate at which AChE can be collected.

References


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Ellman’s Assay of Unpurified Waste Medium

Ellman’s Assay of Purified Waste Medium

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